Apolipoprotein A1 Is a Stronger Prognostic Marker Than Are HDL and LDL Cholesterol for Cardiovascular Disease and Mortality in Elderly Men

Gösta Florvall,1 Samar Basu,2 and Anders Larsson

1Section of Clinical Chemistry, Department of Medical Sciences, and 2Clinical Nutrition and Metabolism, Department of Public Health and Caring Sciences, Uppsala University Hospital, Sweden.

The aim of this study was to compare apolipoprotein A1 (ApoA1) and B (ApoB) with high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) as markers for cardiovascular mortality and morbidity in elderly men. We analyzed serum ApoA1, ApoB, total cholesterol, HDL-C, and LDL-C in a group of 77-year-old men (n = 785). The results were correlated with data from the Swedish cause of death registry. Receiver-operating characteristic curves showed that, of the studied serum markers, ApoA1 was the best predictor for ischemic heart disease mortality (area under the curve = 0.724, 95% confidence interval, 0.691-0.755). There were also significant correlations between the apolipoproteins and other known risk markers for cardiovascular disease such as triglycerides, high-sensitivity C-reactive protein (hsCRP), and cystatin C. Serum ApoA1 is a better risk marker than are ApoB, ApoB/ApoA1 ratio, HDL-C, and LDL-C for cardiovascular disease and mortality in elderly men.

Hypcholesterolemia, hypertriglyceridemia, elevated low-density lipoprotein cholesterol (LDL-C), and low high-density lipoprotein cholesterol (HDL-C) are generally accepted as strong risk factors for cardiovascular disease (CVD) and mortality (1–3). However, results from a number of studies have indicated that apolipoprotein B (ApoB), apolipoprotein A1 (ApoA1), and ApoB/ApoA1 ratio could improve the prediction of CVD and mortality in comparison with total cholesterol, HDL-C, and LDL-C (4,5).

The largest of these studies is the AMORIS (Apolipoprotein-related MOrtality RISk) study, which showed that high levels of ApoB were strongly related to increased CVD risk, whereas ApoA1 had a protective function both in men and women (6). The strongest correlation described in the AMORIS study was that between increased risk of fatal myocardial infarction and the ApoB/ApoA1 ratio (7). Apart from the AMORIS study, there are very few studies on apolipoproteins as risk markers in individuals older than 70 years, and even in the AMORIS study there was only a small fraction of participants who were older than 75 years.

A problem with LDL-C and HDL-C, from a laboratory point of view, is that there is no widely accepted international standard for LDL-C and HDL-C. This makes it difficult to compare assays performed at different laboratories, and it limits the possibility to transfer target values for these analytes from one country to another. This speaks in favor of ApoA1 and ApoB for which there exists an international standardization (8).

Recently, immunological apolipoprotein A1 and B methods have been developed for clinical chemistry analyzers, making the test widely accessible. ApoA1 and ApoB can thus be analyzed with short turnaround times, providing rapid test results at a cost comparable to HDL-C and LDL-C. Thus, it is likely that determination of apolipoproteins as risk markers for CVD will increase over the next decade. It is thus important to compare the predictive value of apolipoproteins with that of traditional markers.

The objective of this study was to compare the correlations of total cholesterol, HDL-C, and LDL-C versus ApoB, ApoA1, and ApoB/ApoA1 ratios as predictors for mortality and ischemic heart disease in a well-defined cohort of elderly men older than 70 years. We also wanted to investigate which of the studied lipoprotein markers showed the strongest correlation with mortality.

METHODS

Study Population

This study included Swedish men, 77 years of age, who were participants in the Uppsala Longitudinal Study of Adult Men (ULSAM) (9). This health survey, to identify risk factors for CVD, started in 1970, when all men born between 1920 and 1924 and living in Uppsala were invited to participate (age 50 years). These men were reinvested at ages 60 and 70. At age 77, 1398 men were invited to participate in a re-investigation, and 839 men participated. The study was approved by the Ethics Committee at Uppsala University, and all participants gave their written consent prior to blood sampling.

Sample Collection and Anthropometric Measurements

Blood samples were drawn from an antecubital vein in the morning after a 12-hour (overnight) fast. The samples were immediately frozen at −70°C until analysis.
Medical History and Medication

A total of 785 samples was available for analysis of ApoA1 and ApoB. Medical history and information on smoking habits and medications were obtained by using a self-administered questionnaire. Information on history of CVD (myocardial infarction, ischemic stroke, or angina pectoris) was obtained from the Swedish Hospital Discharge Registry. Information on mortality was obtained from the Swedish cause of death registry. Mean follow-up from blood sampling was 5 years.

ApoA1, ApoB, HDL-C, LDL-C, Cystatin C, and High-Sensitivity C-Reactive Protein

Serum ApoA1 and ApoB measurements were performed by turbidimetry using an Architect ci8200 (Abbott Laboratories, Abbott Park, IL) and reagents from the same manufacturer (9D92-01 and 9D93-01). The total analytical imprecision for the ApoA1 method was 0.9% at 2.25 g/L and for the ApoB method 1.2% at 1.73 g/L.

Triglycerides and cholesterol were analyzed on a Monarch instrument (Instrumentation Laboratories, Lexington, MA). HDL-C was assayed in the supernatant fraction after precipitation with a heparin/manganese–chloride solution. LDL-C was calculated using Friedewald’s formula: LDL-C = HDL-C – (0.42 × serum triglycerides).

Serum cystatin C and high sensitivity C-reactive protein (CRP) measurements were performed by using latex-enhanced reagent (N Latex Cystatin C and CRP; Dade Behring, Deerfield, IL) using a Behring BN ProSpec analyzer (Dade Behring). The total analytical imprecision for the cystatin C method was 4.8% at 0.56 mg/L and 3.7% at 5.87 mg/L.

Statistical Calculations

Spearman rank calculations were performed with the statistical software package Statistica (StatSoft Inc., Tulsa, OK). Associations between variables were tested with Spearman’s rank correlation analysis as only HDL-C and total cholesterol showed a normal distribution. HsCRP, LDL-C, triglycerides, ApoA1, ApoB, and cystatin C were not normally distributed. The Mann–Whitney U test was used for comparison between the group that died due to ischemic heart disease and the rest of the study group. Receiver operating characteristics (ROC) calculations were performed with MedCalc Software (Mariakerke, Belgium). Values of p < .05 were regarded as statistically significant throughout the study.

RESULTS

Study Group

During the study period 76 men died. Of these men, 19 died due to ischemic heart disease. One hundred fifteen men were treated at a hospital for acute myocardial infarction, and 239 men were treated for other circulatory disorders (diseases of the circulatory system, excluding ischemic heart diseases and cerebrovascular diseases).

There were no significant differences in body mass index (BMI), blood pressure, hemoglobin A1c (HbA1c), glucose, insulin, triglycerides, and hsCRP between the men who died due to ischemic heart disease and the rest of the study group (Table 1).

ApoA1, ApoB, and ApoB/ApoA1 Ratio in the Study Population

Median ApoA1 concentration in the cohort was 1.49 g/L (interquartile range 1.32–1.66 g/L), and mean value was 1.51 g/L. Median ApoB concentration in the cohort was 1.00 g/L (0.87–1.15 g/L), and mean value was 1.01 g/L. Median ApoB/ApoA1 ratio in the cohort was 0.667 (0.564–0.793), and mean ratio was 0.687.

Correlations Between ApoA1, ApoB, and ApoB/ApoA1 Ratio and Other Laboratory Markers

The strongest correlations were observed between ApoA1 and HDL-C (R = .859), ApoB and LDL-C (R = .838), ApoB and total cholesterol (R = .8078), ApoB/ApoA1 ratio and LDL-C (R = .563), and ApoB/ApoA1 ratio and HDL-C (R = .557) (Table 2). ApoA1 (R = −.138) and ApoB/ApoA1 ratio (R = .118) showed significant correlations with the inflammation marker hsCRP. ApoA1 (R = −.1341) and ApoB/ApoA1 ratio (R = .095) showed significant correlations with the glomerular filtration marker cystatin C.

Association Between ApoA1, ApoB, and ApoB/ApoA1 Ratio and Mortality or Hospital Care

The highest area under the curve (AUC) found was between ApoA1 and ischemic heart disease mortality (AUC = 0.724; 95% confidence interval [CI], 0.691–0.755) (Table 3 and Figure 1). ApoA1 was also significantly associated with total mortality (AUC = 0.584; CI, 0.548–0.619), and hospital care due to ischemic heart disease (AUC = 0.561; CI, 0.525–0.596) and other circulatory disorders (AUC = 0.587; CI, 0.552–0.622). HDL-C was also significantly associated with ischemic heart disease mortality (AUC = 0.589;
Table 2. Spearman’s Rank Correlations Between Different Laboratory Markers

<table>
<thead>
<tr>
<th>Laboratory Markers</th>
<th>R</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>ApoA1 and total cholesterol</td>
<td>0.390</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>ApoA1 and HDL cholesterol</td>
<td>0.859</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>ApoA1 and LDL cholesterol</td>
<td>0.203</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>ApoA1 and triglycerides</td>
<td>-0.211</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>ApoA1 and ApoB</td>
<td>0.050</td>
<td>NS</td>
</tr>
<tr>
<td>ApoA1 and hsCRP</td>
<td>-0.138</td>
<td>.0001</td>
</tr>
<tr>
<td>ApoA1 and cystatin C</td>
<td>-0.134</td>
<td>.0002</td>
</tr>
<tr>
<td>ApoB and total cholesterol</td>
<td>0.808</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>ApoB and HDL cholesterol</td>
<td>-0.061</td>
<td>NS</td>
</tr>
<tr>
<td>ApoB and LDL cholesterol</td>
<td>0.838</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>ApoB and triglycerides</td>
<td>0.358</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>ApoB and hsCRP</td>
<td>0.034</td>
<td>NS</td>
</tr>
<tr>
<td>ApoB and cystatin C</td>
<td>0.043</td>
<td>NS</td>
</tr>
<tr>
<td>ApoB/ApoA ratio and total cholesterol</td>
<td>0.420</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>ApoB/ApoA ratio and HDL cholesterol</td>
<td>-0.557</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>ApoB/ApoA ratio and LDL cholesterol</td>
<td>0.563</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>ApoB/ApoA ratio and triglycerides</td>
<td>0.422</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>ApoB/ApoA1 ratio and hsCRP</td>
<td>0.117</td>
<td>.0001</td>
</tr>
<tr>
<td>ApoB/ApoA1 ratio and cystatin C</td>
<td>0.096</td>
<td>.007</td>
</tr>
</tbody>
</table>

Notes: p < .05 is considered significant.

LDL = low-density lipoprotein; hsCRP = high-sensitivity C-reactive protein.

CI, 0.554–0.624). Ischemic heart disease also showed significant correlations with hsCRP (AUC = 0.566; CI, 0.530–0.601) and cystatin C (AUC = 0.644; CI, 0.609–0.677) but not with serum triglycerides. Patients in the 1st, 2nd, 3rd, and 4th ApoA1 quartiles had a risk of subsequent death due to ischemic heart disease of 5.1%, 2.5%, 1.5%, and 0.5% (Figure 2).

Table 3. Characteristics of Receiver Operating Characteristics
Analysis of the Lipoproteins as Prognostic Markers for Ischemic Heart Disease Mortality

<table>
<thead>
<tr>
<th>Markers</th>
<th>AUC</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Criterion</th>
</tr>
</thead>
<tbody>
<tr>
<td>ApoA1</td>
<td>0.724</td>
<td>52.6</td>
<td>82.4</td>
<td>1.28</td>
</tr>
<tr>
<td>ApoB</td>
<td>0.602</td>
<td>47.4</td>
<td>76.8</td>
<td>0.86</td>
</tr>
<tr>
<td>ApoB/A1 ratio</td>
<td>0.530</td>
<td>84.2</td>
<td>29.9</td>
<td>0.585</td>
</tr>
<tr>
<td>hsCRP</td>
<td>0.566</td>
<td>94.7</td>
<td>23.0</td>
<td>0.912</td>
</tr>
<tr>
<td>Cystatin C</td>
<td>0.644</td>
<td>47.4</td>
<td>81.3</td>
<td>1.23</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>0.490</td>
<td>36.8</td>
<td>79.1</td>
<td>1.82</td>
</tr>
</tbody>
</table>

Note: AUC = Area under the curve; Apo = apolipoprotein; HsCRP = high-sensitivity C-reactive protein.

**DISCUSSION**

ApoA exists in two forms: ApoA1 and ApoA2 (10). ApoA1 is essential for the binding of the HDL particles to the ATP-binding cassette transporter (ABCA-1) on the cell surface (11). ApoA1 is also a cofactor for lecithin cholesterol acyl transferase (12,13). The ApoA1 concentration in plasma is usually strongly correlated with the amount of HDL-C (14). ApoB exists in two forms, ApoB-48 and ApoB-100 (15,16). ApoB-48 is synthesized in the intestine and is an important component in chylomicrons that can be found in plasma after a meal (15). In a fasting situation more than 95% of the apolipoprotein in the circulation is ApoB-100. ApoB-100 is synthesized in the liver and is found in very-low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), and LDL particles (16). There is one ApoB molecule per LDL particle, and the presence of the protein is necessary for the binding of the particle to the LDL receptor (17,18). The ApoB concentration in plasma is strongly correlated with the amount of LDL-C (14). This correlation is in agreement with our findings. Thus, ApoA1 and ApoB show good correlation with HDL-C and LDL-C, but in some studies the apolipoproteins have been shown to be more strongly associated with CVD and mortality. The most important risk factor for myocardial infarction in all geographic regions in the INTERHEART study was the ApoB/ApoA1 ratio (19). In contrast, ApoA1 showed the strongest association with fatal and nonfatal cardiovascular events in patients with coronary artery disease (20).

The finding in this study that ApoA1 is the strongest risk marker for CVD in old men and for mortality is in agreement with the increased evidence that HDL-based therapy effectively prevents CVD (21–23). The beneficial effects of ApoA1Milano show the protective role of ApoA1. Treatment with ApoA1Milano combined with phospholipids during periods of ischemia significantly reduced infarct size, and myocardial damage in an animal model (24). Several research groups are working with ApoA1 and ApoA1 mimetic peptides, or are finding other means of increasing the HDL fraction and ApoA1 (25–27). The HDL particle is known mainly for its ability to facilitate reverse cholesterol transport, but it also possess anti-thrombotic, anti-oxidant, anti-inflammatory, and endothelium-stabilizing properties that may benefit atherosclerosis (26,28–30). Both ApoA1 and HDL-C showed significant negative correlations with the glomerular filtration marker cystatin C and the inflammation marker hsCRP in this study. An anti-

![Figure 1. Receiver operating characteristics curve with regard to death due to ischemic heart disease for apolipoprotein A1 with area under the curve of 0.724 (95% confidence interval, 0.691–0.755).](image-url)
inflammatory effect may have a protective effect on the kidney function, but the endothelium-stabilizing properties of the HDL particle may also play a role (31). Reduced kidney function is a predictor of mortality in hypertensive (32) and elderly persons (33) and in patients with myocardial infarction (34).

Conclusion

The association between cardiovascular morbidity and/or mortality and ApoA1 was stronger than that for the other studied markers. This finding is in contrast to those of previous reports showing a stronger association with ApoB/ApoA1 ratio in younger populations. The findings in this study may thus be due to the higher age of the individuals in comparison with the ages of persons in other studies on apolipoproteins as risk markers for cardiovascular morbidity and/or mortality. The association between cardiovascular morbidity and/or mortality and ApoA1 is in agreement with previous studies showing HDL-C superior to LDL-C as a risk marker for coronary artery disease and stroke in an elderly population (35). Another recent study showed an association between ApoA1 and coronary events, but it failed to show any correlation between LDL-C and coronary or cerebrovascular events or any correlation between the achieved LDL-C level and risk reduction in people >70 years of age (36). The same study also failed to show any correlation between ApoB and coronary events. The inverse correlation between HDL-C and cardiovascular events is in concordance with other recent studies (37,38). Although ApoA1 had a statistically significant inverse correlation with study endpoints, it should be pointed out that there was a low order correlation. Thus, the results should be interpreted with caution for individual patients, but the findings raise questions as to which lipoprotein measurements are most appropriate to determine coronary risk in elderly persons and how the efficacy of lipid-lowering drugs should be measured in this patient group.

Acknowledgments

This study was supported financially by the Uppsala University Research Fund.

Address correspondence to Anders Larsson, MD, Department of Medical Sciences, University Hospital, 8-751 85 Uppsala, Sweden. E-mail: anders.larsson@akademiska.se

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Received January 17, 2006
Accepted June 29, 2006
Decision Editor: Huber R. Warner, PhD